

1. *Journal of the American Medical Association*, 1997; 278: 1000-1005.

- Hepatitis C virus (HCV) is a common blood-borne pathogen annually infecting three to four million people worldwide. Currently, an estimated 170 million people are infected globally, representing a nearly 5-fold greater prevalence than human immunodeficiency virus.¹

- The current standard-of-care therapy, a combination of pegylated interferon and ribavirin, is effective in only 30% of patients infected with genotype 1 HCV and is associated with significant side effects. Thus, there remains a need for new, more effective and better tolerated HCV treatment options.
- The HCV polymerase has been an attractive antiviral target. Nucleoside analogs, or more recently non-nucleosides such as IDX184, target the active site of the enzyme¹, while multiple classes of non-nucleoside polymerase inhibitors (NNIs) target different allosteric sites in the enzyme.
- IDX375 is a novel NNI developmental candidate that targets the palm pocket of the NS5B polymerase.
- This study evaluated the *in vitro* biochemical and cell-based activities of IDX375, and its pharmacokinetic profile in the rat and the monkey.

Biochemical assays: K_{m} , inhibitory constant (K_i) and Michaelis constants (K_{m}) were determined by standard methods. Human polymerase activity was determined by measuring the incorporation of α - ^{32}P dNMP using activated calf thymus DNA as template.

HCV replicon assay: Huh-7 cells stably expressing a replicon containing the luciferase transgene were seeded onto 96-well plates, cultured for 3 days in the presence of drug and subjected to a luciferase assay. Cytotoxicity was measured by MTS in GSA-1. Huh-7 or HepG2 cells after 3 or 9 days of treatment.

HCV *in vitro* infection assays: HFC cells were infected with JFH-1 HCV (genotype 1a) and treated with serial drug dilutions. After 16 hours, virus inoculum was removed and cultures were incubated with drug for 3 days. Drug susceptibility was determined by ELISA using anti-HCV core antibody.

Long-term treatment assay: GS4-1 cells stably expressing a bicistronic HCV replicon were cultured in the presence of drug, but without G418, for 14 days. Cells were split, RNA was collected every 3–4 days and replicon RNA levels were measured by RT-qPCR of the HCV 5'-UTR and normalized to GAPDH. Following 14-day treatment, cells were seeded into 10 cm dishes in the absence of compound ± G418 for 21 days to determine the presence of the HCV replicon. The number of colonies in each plate was counted following staining with crystal violet in 50% ethanol.

Monkey and rat pharmacokinetics (PK): For the PK studies, serial plasma samples were obtained from male Sprague-Dawley rats and cynomolgus monkeys given single doses of IDK375 (2 mg/kg IV or 10 mg/kg PO, 2 animals per dose group). For tissue studies, samples of plasma and tissue were obtained from 2 cynomolgus monkeys. All samples were treated with acidified acetonitrile and concentrations of IDK375 were determined by reversed-phase LC-MS/MS.

Biochemical characterization of IPV-202

	HCVts	DNA pol α	DNA pol β	DNA pol γ	RNA pol II
K_m (μM)	0.030	0.018	> 100	> 100	> 100

As shown in **Table 1**, IDX375 inhibited HCV polymerases of genotypes 1a and 1b with submicromolar IC_{50} values, but did not inhibit human DNA polymerases α , β or γ or human RNA polymerase II.

- In biochemical experiments (data not shown), the choice of γ -NTP substrate did not affect the IC_{50} of IDX375; values ranged from 1.4 (Δ ADP, ATP) to 4.0 (Δ ATP, GTP).

- Kinetic analyses (data not shown) with the 1b HCV polymerase determined the K_m of IDX375 to be 40 nM, similar to the K_m values of the 4 nucleotides.
 - IDX375 was found to be a noncompetitive inhibitor with respect to the 4 nucleotide substrates.
 - Inhibition was mixed with respect to RNA template.

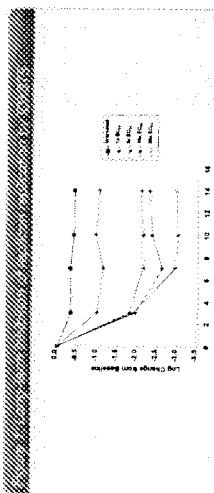
Table 2. Activity of IDJX375 in a standard HCV genotype 1b replicon assay

	6	2.3	5	>100	>43,000
Luciferase reporter-based assay	+	+	+	+	+
EC ₅₀ (μM)	6	2.3	5	>100	>43,000

- As seen in **Table 2**, IDX375 is a potent inhibitor of genotype 1b HCV replication with low cytotoxicity and excellent selectivity.
- In a variety of cell lines, IDX375 showed negligible cytotoxicity; in 9-day assays the CC_{50} of IDX375 was 88 μ M in Huh-7 cells and >100 μ M in HepG2 cells.

- The presence of 45% human serum increased the EC_{50} of ID375 by 25-fold in the 1b luciferase replicon assay.
- The activity of ID375 against the JFH-1 genotype 2a virus was lower, $EC_{50} = 18.4 \mu M$, using the core ELISA assay.

- As shown in **Table 3** and **Figure 1**, longer term treatment with IDX375 achieved a 1.0 log₁₀ reduction in replicon RNA at a 1x EC₅₀ of IDX375 and a 3 log₁₀ reduction at 20x EC₅₀ of IDX375.



	-	0.42
	1	1.03
	5	2.05
	10	2.29
	20	2.86

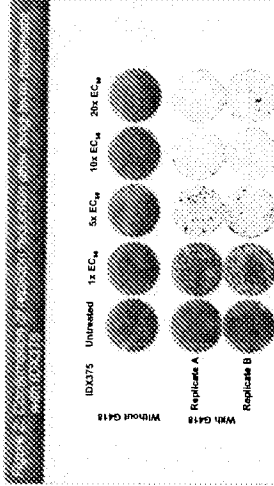
Values represent log₂ reduction derived from averages from four independent experiments. Log₂ reduction was calculated by subtracting the average log₂ copies HCV/3APDH RNA of the sample at Day 14 from the average log₂ copies HCV/3APDH RNA of the untreated control at Day 0.

- ID375 is a potent and selective noncompetitive inhibitor that targets the palm domain of the HCV NS5B enzyme.
- ID375 inhibited HCV replication in an *in vitro* replicon assay with an EC_{50} value of 2.3 nM and a selectivity index of >43,000

- ID3375 was not cytotoxic in test cell lines.
- Treatment of replicon cells with 20x EC_{50} of ID3375 for 14 days resulted in a 3 log₁₀ reduction in HCV replicon RNA and reduced the number of replicon-containing foci in cell culture.
- The PK profile of ID3375 in the rat and the cynomolgus monkey shows adequate drug exposure in the systemic circulation. Moreover, the drug selectively concentrated in the liver.
- The preclinical PK profile of ID3375 suggests the potential for once-a-day dosing. After 24 h, plasma levels remained 10- to 30-fold above the EC_{50} in both rats and monkeys given single 10 mg/kg oral doses.
- Based on the *in vitro* antiviral potency and the exposure seen in animal PK studies, ID3375 is a promising candidate for clinical evaluation.

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1. Wasley A and Alter MJ (2000). Semin. Liver Dis. 20:1-16.
2. Cretton-Scott E, et al (2008). J Hepatology 48, S220.
3. Ståhlbrand D, Lanford R, Cretton-Scott E, et al (2008). J Hepatology 48, S20.



Quantitation of colonies shown above

[illegible]

- As seen in **Figure 2**, the number of replicon colonies was reduced in a dose-dependent manner after 14-day treatment with IDX375.
- At 1x EC₅₀, the reduction in colonies was already visible and became marked at $\geq 5x$ EC₅₀.

- Since the submission of the abstract, the PK profile of IDX375 has been refined in more detailed studies. The recent data are presented below.

Table 4: Plasma PK profile of IDX375 in the rat and monkey

Concentration	Parameter	Pre-exposure	Post-exposure
2 mg/kg IV	Cl (L/h/kg)	2.5	0.24
	Vd (L/kg)	17	0.77
	$T_{1/2}$ (h)	4.6	2.3
	C_{max} (nM)	860	4160
10 mg/kg PO	T_{max} (h)	0.5-4.0	4.0
	Bioavailability (%)	101	28

- The oral bioavailability of IDX375 was good to excellent in rats and monkeys.